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Proc Natl Acad Sci U S A 1982 Nov;**79(22)**:6777-6781

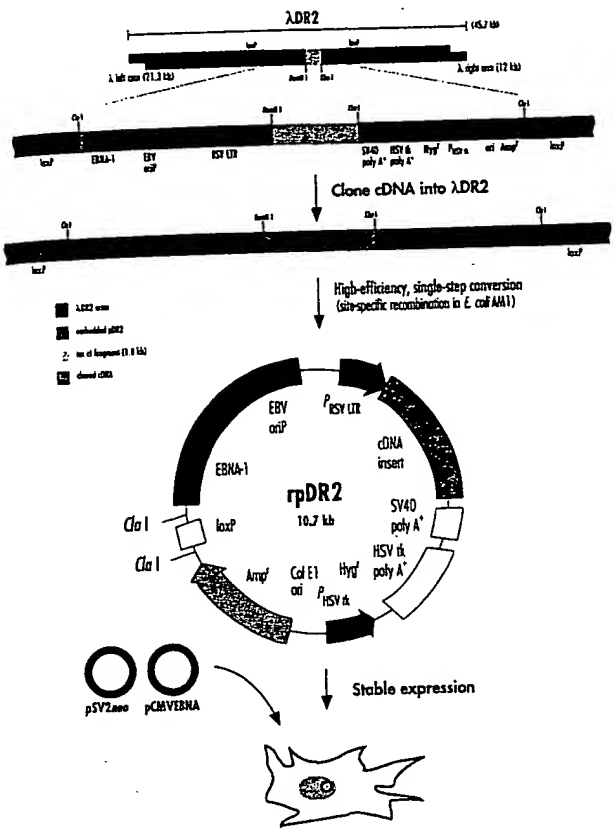
The Rous sarcoma virus long terminal repeat is a strong promoter when introduced into a variety of eukaryotic cells by DNA-mediated transfection.

Gorman CM, Merlino GT, Willingham MC, Pastan I, Howard BH

We characterized the transcriptional activity of the long terminal repeat (LTR) of Rous sarcoma virus by constructing a recombinant plasmid, pRSVcat, in which bacterial chloramphenicol acetyltransferase (CAT; acetyl-CoA:chloramphenicol 3-O-acetyltransferase, EC 2.3.1.28) coding sequences are placed under LTR control. We find that the LTR directs relatively high levels of CAT synthesis within 48 hr after calcium phosphate-mediated introduction of this plasmid into CV-1 monkey kidney cells, chicken embryo fibroblasts, Chinese hamster ovary cells, HeLa cells, or mouse NIH/3T3 cells. The level of CAT synthesis is 3-fold higher in CV-1 cells and up to 10-fold higher in HeLa and mouse NIH/3T3 cells than after transfection with a related vector, pSV2cat, carrying CAT sequences under control of the simian virus 40 early promoter. We have shown, by primer extension, that the amounts of CAT-specific mRNAs encoded by pRSVcat and pSV2cat correlate with the levels of CAT enzyme activity. By both S1 nuclease mapping and primer extension, we have demonstrated that the start site for RNA transcription within the LTR of pRSVcat corresponds to previous mapping data. We estimated transfection efficiencies by monitoring immunofluorescence induced by a rhodamine-labeled CAT antibody. Our results indicate that the Rous sarcoma virus LTR can direct synthesis of high levels of functional mRNA and has a wide expression range. The observed high transcriptional activity of the LTR is significant because it has been postulated that this LTR promotes activity of adjacent cellular oncogenes.

PMID: 6294651, UI: 83091044

λDR2 Cloning and Expression



Product	Size	Cat. #
λDR2 arms (*Bam*H I/*Xba* I digested), dephosphorylated	10 µg	6168-1
λDR2 Cloning & Expression Kit	each	K1450-1

UNIQUE CLONING SITES

*Bam*H I, *Xba* I

VECTOR SIZE: 45.7 kb (λDR2)

KIT COMPONENTS

- λDR2 arms
- pSV2*neo*
- pCMVEBNA
- Test insert
- *E. coli* AM1
- *E. coli* K802
- 23-mer sequencing primer
- 21-mer sequencing primer
- Complete User Manual *(PT1011-1)*
- pDR2 Sequence Information *(PT2006-5)*

STORAGE CONDITIONS

–20°C

4°C for short-term storage (daily/weekly use) of all items except *E. coli*

DESCRIPTION

λDR2 is a lambda phage containing an embedded version of the Epstein-Barr virus (EBV) shuttle vector pDR2, which provides the elements necessary for stable expression in permissive human cell lines. cDNA can be cloned with high efficiency, converted to plasmid form (either individually or as a library), and transfected into human cells. Thus, λDR2 can be used to isolate cDNAs that impart a selective phenotype to a particular human cell line.

λDR2 uses the tac-cI selection system (1) to allow construction of libraries with essentially 100% recombinants. cDNA libraries with greater than 10^6 independent clones can be generated and inserts up to 7.0 kb in length can be accepted. λDR2 also contains *lox*P sites flanking the embedded pDR2 to allow one-step phage-to-plasmid conversion. The *lox*P sites undergo site-specific recombination when transfected into an *E. coli* strain with *Cre* recombinase (e.g., AM1). pDR2 plasmids carrying cloned inserts are effectively popped out into circular plasmid form ready for sequencing or expression in human cells.

λDR2 is available as *Bam*H I/*Xba* I-digested arms. pDR2 is also available separately (see following page). *E. coli* host cells, a test insert, vector maps, and a complete User Manual are provided with the kit and λDR2 arms. The λDR2 Cloning and Expression Kit includes sufficient reagents for 3–4 cloning experiments.

Premade cDNA libraries in λDR2 and pDR2 are available (see CH 15).

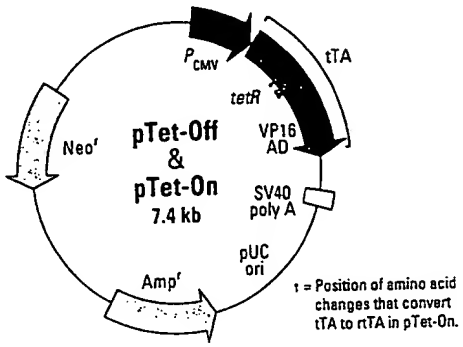
REFERENCES

1. Murphy, A. & Schimke, R. (1991) *Nucleic Acids Res.* 19:3403.
2. Murphy, A. J. M., *et al.* (1992) *METHODS* 4:111–131.
3. Swirski, R. A., *et al.* (1992) *METHODS* 4:133–142.

EXHIBIT B Page 1 of 2

Annex 2

Tet-Off™ & pTet-On™ Vectors



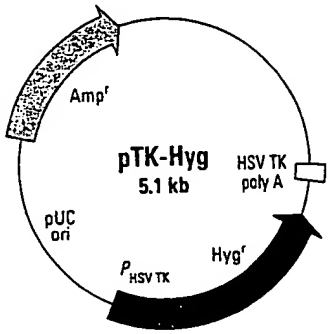
	Product	Size	Cat. #
NEW	pTet-Off Vector	20 µg	K1620-A
NEW	pTet-On Vector	20 µg	K1621-A

Mammalian expression vectors which express the Tc-responsive transcriptional activator (tTA) or the reverse tTA (rtTA) from the constitutive, strong immediate early promoter of cytomegalovirus (P_{CMV}). tTA, which is expressed from pTet-Off, is a fusion of amino acids 1–207 of the tetracycline repressor (TetR) and the negatively charged C-terminal activation domain (130 amino acids) of the VP16 protein of herpes simplex virus. pTet-On is similar to pTet-Off except for four amino acid changes that convert the TetR to a rTetR, and consequently, tTA to rtTA. pTet-Off was originally described as pUHD15-1*neo* by Resnitzky *et al.* (1) and was created by insertion of a neomycin resistance gene into pUHD15-1 (2). pTet-On was originally described as pUHD17-1*neo* by Gossen *et al.* (3). pTet-On can be distinguished from pTet-Off by digestion with *Hind* III.

pTet-Off and pTet-On are provided with complete vector information packets (PT3073-5 and PT3072-5, respectively).

GENBANK ACCESSION #: U89929 pTet-Off
U89930 pTet-On

pTK-Hyg Selection Vector



	Product	Size	Cat. #
NEW	pTK-Hyg Vector	10 µg	6153-1

Selection vector which confers hygromycin resistance in mammalian cells for the selection of stably transformed cells using hygromycin. pTK-Hyg is especially useful for selection of double-stable cell lines using the Tet-Off™ or Tet-On™ Gene Expression Systems. The HSV TK promoter lacks an enhancer element reducing the unwanted activation of pTRE- and pBI-derived plasmids upon cointegration into the host cell's genome. pTK-Hyg contains the pUC origin of replication and ampicillin resistance gene for propagation and selection, respectively, in *E. coli*.

pTK-Hyg is provided with a complete vector information packet (PT3082-5).

GENBANK ACCESSION #: U40398

REFERENCES

1. Resnitzky, D., *et al.* (1994) *Mol. Cell. Biol.* 14:1669-1679.
2. Gossen, M. & Bujard, H. (1992) *Proc. Natl. Acad. Sci. USA* 89:5547–5551.
3. Gossen, M., *et al.* (1995) *Science* 268:1766–1769.

EXHIBIT
B
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IPEA/ EP

PCT

DEMAND

under Article 31 of the Patent Cooperation Treaty:
The undersigned requests that the international application specified below be the subject of international preliminary examination according to the Patent Cooperation Treaty.

For International Preliminary Examining Authority use only

Identification of IPEA	Date of receipt of DEMAND

Box No. I IDENTIFICATION OF THE INTERNATIONAL APPLICATION		Applicant's or agent's file reference
International application No. PCT/EP95/03445	International filing date *(day/month/year)* 1 September 1995 (01.09.1995)	(Earliest) Priority date *(day/month/year)* 2 September 1994 (02.09.1994)
Title of invention NON SELF INACTIVATING, EXPRESSION TARGETED RETROVIRAL VECTORS		

Box No. II APPLICANT(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*	Telephone No.:
GSF - FORSCHUNGSZENTRUM FÜR UMWELT UND GESUNDHEIT GMBH Ingolstädter Landstr. 1, Neuherberg D-85764 Oberschleissheim DE	Facsimile No.: Teleprinter No.:

State *(i.e. country)* of nationality:	State *(i.e. country)* of residence:
DE	DE

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

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GB	DE

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

SALLER, Robert Michael
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DE

State *(i.e. country)* of nationality:	State *(i.e. country)* of residence:
DE	DE

☐ Further applicants are indicated on a continuation sheet.

International application No.
PCT/EP95/03445

Box No. III AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The following person is [XX] agent [ ] common representative

and [XX] has been appointed earlier and represents the applicant(s) also for international preliminary examination.

[ ] is hereby appointed and any earlier appointment of (an) agent(s)/common representative is hereby revoked.

[ ] is hereby appointed, specifically for the procedure before the International Preliminary Examining Authority, in addition to the agent(s)/common representative appointed earlier.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

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Patentanwälte
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Telephone No.: 089 / 38 16 10 0

Facsimile No.: 089 / 340 14 79

Teleprinter No.:

[ ] Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Box No. IV STATEMENT CONCERNING AMENDMENTS

The applicant wishes the International Preliminary Examining Authority*

(i) [X] to start the international preliminary examination on the basis of the international application as originally filed.

(ii) [ ] to take into account the amendments under Article 34 of

- [ ] the description (amendments attached).
- [ ] the claims (amendments attached).
- [ ] the drawings (amendments attached).

(iii) [ ] to take into account any amendments of the claims under Article 19 filed with the International Bureau (a copy is attached).

(iv) [ ] to disregard any amendments of the claims made under Article 19 and to consider them as reversed.

(v) [ ] to postpone the start of the international preliminary examination until the expiration of 20 months from the priority date unless that Authority receives a copy of any amendments made under Article 19 or a notice from the applicant that he does not wish to make such amendments (Rule 69.1(d)). *(This check-box may be marked only where the time limit under Article 19 has not yet expired.)*

* Where no check-box is marked, international preliminary examination will start on the basis of the international application as originally filed or, where a copy of amendments to the claims under Article 19 and/or amendments of the international application under Article 34 are received by the International Preliminary Examining Authority before it has begun to draw up a written opinion or the international preliminary examination report, as so amended.

Box No. V ELECTION OF STATES

[X] The applicant hereby elects all eligible States *(that is, all States which have been designated and which are bound by Chapter II of the PCT)* except .....

(If the applicant does not wish to elect certain eligible States, the name(s) or country code(s) of those States must be indicated above.)

Internati nal application No.
PCT/EP95/03445

Box No. VI CHECK LIST

The demand is accompanied by the following documents for the purposes of international preliminary examinati n:

			For International Preliminary Examining Authority use only: received	not received
1. amendments under Article 34				
description	:	sheets	☐	☐
claims	:	sheets	☐	☐
drawings	:	sheets	☐	☐
2. letter accompanying amendments under Article 34	:	sheets	☐	☐
3. copy of amendments under Article 19	:	sheets	☐	☐
4. copy of statement under Article 19	:	sheets	☐	☐
5. other *(specify)*:	:	sheets	☐	☐

The demand is also accompanied by the item(s) marked below:

1. ☐ separate signed power of attorney
2. ☐ copy of general power of attorney
3. ☐ statement explaining lack of signature
4. ☒ fee calculation sheet
5. ☒ other *(specify)*:
   Check Deutsche Bank
   No. 33974967 DM 3270,--

Box No. VII SIGNATURE OF APPLICANT, AGENT OR COMMON REPRESENTATIVE

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the demand).

Werner Behnisch

Attorney for Applicant
Dr. Werner BEHNISCH

München, 11 September 1995 (11.09.1995)

For International Preliminary Examining Authority use only

1. Date of actual receipt of DEMAND:

2. Adjusted date of receipt of demand due to CORRECTIONS under Rule 60.1(b):

3. ☐ The date of receipt of the demand is AFTER the expiration of 19 months from the priority date and item 4 or 5, below, does not apply. ☐ The applicant has been informed accordingly.

4. ☐ The date of receipt of the demand is WITHIN the period of 19 months from the priority date as extended by virtue of Rule 80.5.

5. ☐ Although the date of receipt of the demand is after the expiration of 19 months from the priority date, the delay in arrival is EXCUSED pursuant to Rule 82.

For International Bureau use only

Demand received from IPEA on:

PCT

FEE CALCULATION SHEET

Annex to the Demand for internati nal preliminary examination

International application No. PCT/EP95/03445	For International Preliminary Examining Authority use only
Applicant's or agent's file reference P 7607 mg	Date stamp of the IPEA

Applicant GSF - FORSCHUNGSZENTRUM FÜR UMWELT UND GESUNDHEIT GMBH
Ingolstädter Landstr. 1, Neuherberg, D-85764 Ober-
schleissheim, DE - et al

Calculation of prescribed fees

1. Preliminary examination fee	DEM 3000	P
2. Handling fee	DEM 270	H
3. Total of prescribed fees Add the amounts entered at P and H and enter total in the TOTAL box	DEM 3270 TOTAL	

Mode of Payment

- ☐ authorization to charge deposit account with the IPEA (see below)
- ☒ cheque No.33974967 Deutsche Bank
- ☐ postal money order
- ☐ bank draft
- ☐ cash
- ☐ revenue stamps
- ☐ coupons
- ☐ other *(specify)*:

Deposit Account Authorization *(this mode of payment may not be available at all IPEAs)*

The IPEA/ _____ ☐ is hereby authorized to charge the total fees indicated above to my deposit account.

☐ *(this check-box may be marked only if the conditions for deposit accounts of the IPEA so permit)* is hereby authorized to charge any deficiency or credit any overpayment in the total fees indicated above to my deposit account.

Deposit Account Number _____ Date *(day/month/year)* _____ Signature _____

See Notes to the fee calculation sheet